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| <b>(54) Title:</b> ELECTROCHEMICAL DIAGNOSTIC DETECTION DEVICE<br><br><b>(57) Abstract</b><br><br><p>An electrochemical diagnostic device and a method for conducting diagnostic tests in clinical, veterinary, industrial and laboratory applications comprises a fibrous matrix feeder strip (2, 13) contacting at one end a sample holder (1) and incorporating optional filter elements (3-5), the other end being contactable with working (16), reference (19) and auxiliary electrodes (10) and reagent carriers (12, 15). Sample is transferred by capillary action from the sample holder along the feeder strip to allow a reaction between the target analyte and reagents to take place and also allowing electrical conductivity between the electrodes. The reaction end product is measured by amperometric, coulometric or other voltammetric means.</p> |           |  |

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## **ELECTROCHEMICAL DIAGNOSTIC DETECTION DEVICE**

The present invention relates to a device and a method for conducting diagnostic tests in which a liquid sample is transferred by capillary action through a fibrous matrix for electrochemical detection. The invention relates in particular to clinical and veterinary tests, but is equally useful in industrial or laboratory applications.

Over the last 20 years or so, diagnostic tests have been adapted to various rapid test kit formats so as to make them suitable for use in decentralised healthcare sites such as physicians' offices or small laboratories, or for self-testing. The performance of tests at these sites is beneficial in that it eliminates the need to transport samples to a central laboratory. These tests are generally designed as single use disposable kits in which the procedures, equipment and operator training requirements are simplified, and the reaction time may also be reduced. These factors combine to reduce the overall time to obtain a result from such tests, thereby making them relatively quick to conduct.

Quantitative rapid test kits are designed to be processed and read by small specifically designed instruments. Many rapid test kit formats designed for such instruments can perform only a limited selection of the range of test categories suitable for decentralised use. This includes clinical chemistry and the cholesterol sub-fractions, immunoassays, and DNA probes. DNA probes are particularly useful for decentralised use in the identification of causative agents in infections. Clearly, the capability to perform a wide range of test categories is a desirable attribute for a rapid test kit and instrument format designed for decentralised use.

There have recently been developed some rapid test kit formats using electrochemical, rather than the more widely used photometric detection. This method of detection has the advantage of offering various electroanalytical methods, some of these requiring only simple electronic instrumentation.

One such electroanalytical method is amperometric detection. In this method, the current resulting from a redox reaction of a suitable chemical mediator at an electrode is measured. The mediator can be the end product of a reaction sequence involving the analyte. The electrode at which the mediator redox reaction takes place is the working electrode, which is held at a potential relative to that of a reference electrode. The working electrode

potential is sufficiently positive or negative to allow the redox reaction at the electrode to proceed at its maximal rate. Current passes between the working electrode and an auxiliary electrode, with the aid of supporting electrolyte such as potassium chloride.

5 The reference electrode potential is held constant by an equilibrium set up between a metal such as silver and its sparingly soluble salt, silver chloride. The reference electrode can also serve as the auxiliary electrode by allowing current to flow through it in a two electrode system, or the auxiliary electrode can be used as a separate electrode. The two electrode system is disadvantageous in that current flowing through the reference electrode can cause a change in its equilibrium potential.

10 The current obtained in amperometric detection typically displays a continuous decay caused by an expanding diffusion layer of product formation slowing the diffusion of reactant to the electrode surface. It is known that a steady state current can be obtained by placing a working and an auxiliary electrode in close proximity to and facing each other, so as to limit the expansion of the diffusion layers. This can also be achieved by an  
15 interdigitating array of working and auxiliary electrode surfaces (J. Electroanal. Chem., (1965) 10 295 and 538). Allowing the current to reach a steady state has the advantage that the time at which the current is read is not critical.

A device based on amperometric detection for determining glucose, cholesterol and other analytes in whole blood is disclosed in EP 0 230 786. The device comprises co-planar  
20 reference and working electrodes, the working electrode having enzyme, electrochemical mediator and other reagents incorporated in it by a screen printing process. In this device, the electrodes are used in a two electrode system, and an integrated series of measurements are required to obtain a single value for digital display. The device does not incorporate any specific method or structure for analyte separation and could not be used  
25 for the combined separation and detection of the cholesterol sub-fractions, for example.

An electrochemical capillary fill device has been developed by Birch et al (Electroanalysis (1992) 4:1-9; EP Applic. No. 87306513.0). Sample is introduced by capillary action to a rectangular electrochemical cell in which enzyme and electrochemical mediator are deposited. After allowing the enzyme reaction to go to completion, the oxidised or reduced  
30 mediator is electrolysed and measured coulometrically by integrating the resulting current

over a period of time. This device is superior in some ways to the two-electrode amperometric device described in EP 0 230 786, but it similarly does not incorporate any specific method or structure for analyte separation.

Cholesterol measurement has been recognised as a particularly useful application for kit formats for monitoring cholesterol levels in the prevention and treatment of heart disease. The cholesterol sub-fractions, high and low, density lipoprotein cholesterol (HDL-cholesterol and LDL-cholesterol) which are now also recognised as further indicators have also recently been adapted to various rapid test kit formats. These have adapted the standard procedures for the measurement of these sub-fractions which involves incubation of a serum or plasma sample with a precipitation reagent, which can either precipitate all but the required fraction, or the required fraction itself, and centrifuging the resulting precipitate. The required sub-fraction level can be obtained either directly from measuring the cholesterol sub-fraction concentration in the remaining supernatant or by a calculation involving the total cholesterol concentration.

Rittersdorf et al (CA 2,023,926) for example, adapted a method for the determination of HDL cholesterol to a dry reagent reflectance photometry strip format. In this method, sample passes through a fibrous carrier containing the precipitating agent, a further carrier which filters out the precipitate, and is brought into contact with a reflectance reagent zone where the remaining HDL-cholesterol is determined by reflectance photometry. The complex design of the test strip complicates the development of further test types such as immunoassay tests. Also, the instrumentation is not economical and is designed to carry out only one test at a time.

Some immunoassay rapid test kits have been developed using methods to separate antibody-antigen complexes as immunoprecipitates. Rapid immunoassay test kits are useful for applications such as pregnancy, ovulation, CK-MB (for cardiac infarct diagnosis) etc. Ollington et al (PCT/US91/02919) have developed a disposable immunoassay reaction device for the direct determination of LDL cholesterol and other immunoassay tests. An immunoprecipitate of HDL and VLDL sub-fractions with antibody-coated latex beads is filtered out with the aid of a centrifuge, but a means of measuring the target analyte is not incorporated in the device.

The observation that a solution of an electrochemically active species in a solution soaking a paper matrix displays voltammetric characteristics which are comparable to those displayed using conventional techniques is known (J. Electroanal. Chem., (1989) 270:473-8). This prior art does not include the use of a specific structure or device for analyte  
5 separation and electrochemical detection.

Accordingly, the present invention provides a device for conducting a diagnostic test in clinical, veterinary, industrial or laboratory applications on a sample comprising an analyte which is capable of reacting with a reagent in a reaction or reaction sequence to produce an electrochemically detectable end-product, optionally via an intermediate-product, the  
10 device comprising an elongate fibrous carrier matrix connectable with a reference and an auxiliary electrode having a receiving zone for receiving the sample, analyte intermediate- or end-product in a liquid medium and a detection zone to which the sample, analyte, intermediate- or end-product is transferred by capillary action, and a working electrode contactable in use with the detection zone, for electrochemically detecting the end-product  
15 by amperometric, coulometric or other voltammetric methods, when electrical conductivity between the electrodes through the liquid medium and wet matrix is established, the device also including a reagent for reacting with the sample, analyte or the intermediate-product to produce the end-product in the case that the sample, analyte or the intermediate-product is received on the receiving zone of the fibrous carrier matrix.

20 The invention also provides a method for conducting a clinical, veterinary, industrial or laboratory diagnostic test, comprising reacting an analyte in a sample with a reagent in a reaction or reaction sequence to produce an electrochemically detectable end-product, optionally via an intermediate-product, applying the sample, analyte, intermediate- or end-product in a liquid medium to a receiving zone of an elongate fibrous carrier matrix, in the  
25 case of the receiving zone of the fibrous carrier matrix receiving the sample, analyte, or intermediate-product, reacting the sample, analyte or the intermediate product with a reagent included in the device to produce an electrochemically detectable end-product, connecting the fibrous carrier matrix with a reference and an auxiliary electrode, and transferring the sample, analyte, intermediate- or end-product by capillary action to a  
30 detection zone on the fibrous matrix, contacting the detection zone with a working

electrode and electrochemically detecting the end product by amperometric, coulometric or other voltammetric methods, when electrical conductivity between the electrodes through the liquid medium and wet matrix is established.

The device can comprise two or more connectable fibrous carrier matrices of the same or different composition, in linear or branched arrangements, at least one of the fibrous matrices having a detection zone contactable with a working electrode, allowing for example, increased sample flow through a detection zone by increasing the absorbency distal to it, or multiple detections of analytes, calibrators and control samples on branched fibrous matrices.

- 10 The sample may comprise more than one analyte, intermediate- or end-product which can be received on the receiving zone.

- 15 Preferably, the fibrous matrix can effect one or more separation steps in which an analyte, or an interfering analyte, or an intermediate or end product can be retained during transit through the fibrous matrix. This can be achieved for example by using a fibrous matrix of suitable particle retention size to retain colloidal or suspended particles such as for example, lipoprotein-cholesterol precipitates or agglutinated polyclonal antigen-antibody latex particles or red blood cells etc., or for example by incorporating an ion-exchange resin or a ligand or receptor, such as an antibody, antigen, a DNA strand etc. into the fibrous matrix.

- 20 An intermediate or end product received on the receiving zone or transferred to the detection zone can comprise for example a labeled ligand-receptor complex such as a labeled antibody-antigen, DNA-DNA or DNA-RNA complex, an end product comprising a ligand-receptor complex with an electrochemically detectable label, and an intermediate product comprising a ligand-receptor complex with a label which is capable of reacting  
25 with a reagent in a reaction or reaction sequence to produce an electrochemically detectable end product.

Two or more different ligand-receptor complexes having the same label, could be detected in different detection zones, or, having different electrochemically detectable labels, by applying different potentials in the same detection zone.

The detection zone can optionally include a zone on the fibrous matrix contacting in use, a piece, or a stack of two or more pieces of porous or absorbent material, to which the working, reference or auxiliary electrodes are optionally contactable.

5 The sample receiving zone for receiving the sample can optionally contact a sample holder capable of holding the sample such as a hydrophobic gauze, or a receptacle or sample well, or a device capable of carrying out various sample pre-treatment processes, such as for example, a centrifuge device, a sample dispenser, or a device for transferring sample through several chambers or compartments to allow different sample pre-treatment reactions to take place.

10 A reagent, or a background reagent such as buffer, supporting electrolyte, or surfactant necessary for producing or detecting the end product can be optionally carried in a carrier holder contactable with the fibrous matrix, such as a sample well, or on a fibrous carrier matrix, on an electrode or incorporated into the electrode material, such as by spin coating or by a screen printing process or on a piece of porous or absorbent material, or on a piece  
15 of membranous material contactable with the fibrous matrix or working, reference or auxiliary electrode.

The electrodes, reagents and optional pieces of porous or absorbent material are contactable with the sample, analyte, or the intermediate or end product on the fibrous carrier matrix, as a result of both or either, a fixed configuration, comprising of for  
20 example, the working, reference or auxiliary electrode being attached to, or screen printed onto, the fibrous matrix, or by mechanical means such as a device amenable to electromechanical automation.

The electrodes can be arranged so as to allow electrical conductivity through the liquid medium and wet matrix between a different auxiliary, reference or reference/auxiliary  
25 electrode and each of the one or more working electrodes, or between a common auxiliary, reference or reference/auxiliary electrode and two or more working electrodes, the reference, auxiliary or reference/auxiliary electrodes connectable with the fibrous carrier matrix either directly or through one or more pieces of porous or absorbent material or through the sample liquid in the optional sample well.

The working and auxiliary electrodes are made of preferably screen printed graphite ink, or graphite foil, or other material suitable for use as working and auxiliary electrodes. The reference electrode is made of preferably silver/ silver chloride screen printed ink, or other material suitable for use as reference electrodes.

- 5 The use of a fibrous matrix to transport a sample, or an analyte, or an intermediate or an end product by capillary action in the invention has a number of advantages particularly in comparison with the use of capillary action without the assistance of a fibrous matrix. It provides a simple means by which the electrodes in various preferred electrode configurations can be spatially separated and structurally supported and by which a sample,
- 10 or an analyte, or an intermediate or an end product can be brought into contact with a working electrode; it provides a simple means of solution containment and sample dispersion across the electrode surfaces in such preferred electrode configurations; and in some arrangements of the invention it provides a simple means of carrying reagents on absorbent fibrous reagent carriers.
- 15 The use of a fibrous matrix for example allows the use of a three electrode configuration for steady state amperometric detection in which the working and auxiliary electrodes can be placed on opposite sides or in interdigital arrays on one or both sides of the fibrous matrix together with a reference electrode on one or other of the sides.

- The invention allows the use of a fibrous matrix preferably but not necessarily, as a means
- 20 of providing one or more separation steps in which an analyte, or interfering analyte, or an intermediate or end product can be retained during transit through the fibrous matrix. This can be achieved either by using a fibrous matrix of suitable particle retention size to retain colloidal or suspended particles such as for example, lipoprotein-cholesterol precipitate or agglutinated polyclonal antigen-antibody latex particles or red blood cells, or by
- 25 incorporating an ion-exchange resin or a ligand or receptor, such as an antibody, antigen, a DNA strand etc. into the fibrous matrix.

The invention also provides a kit for conducting a clinical, veterinary, industrial or laboratory diagnostic test, the kit including a device of the type described above, together with the reagent or reagents necessary for performing the test.

Conveniently, the kit performs a test which is a human or veterinary clinical chemistry test, or a homogeneous or heterogeneous ligand-receptor binding assay such as an immunoassay or DNA probe test. Commonly conducted tests include blood total cholesterol and/or  
5 cholesterol sub-fraction tests, hormone level tests and tests for one or more infectious agents or one or more antibodies raised in the human or animal body against an infectious agent.

The use of a kit with uniform design for a wide range of different diagnostic tests, whether medical, industrial or experimental, with or without a separation step is also advantageous  
10 in providing a simple instrumentation design.

Electrochemical detection methods in general require simpler detection components, comprising the electrodes and associated potentiostat, voltmeter and other simple electronic circuitry, compared to photometric methods. In amperometric detection, for example, the end product is detected by an electrochemical reaction by poisoning an electrode  
15 at a suitable potential. The potential can be easily varied to suit different reaction end products simply by adjusting the potentiostat. In contrast, photometric detection methods require light emitting diodes for which various monochromators or filters are required for different wavelength emittances, in addition to photo-electric detection requirements. The simpler electronic requirements of electrochemical methods are particularly advantageous  
20 to the present invention in that multiple test modules can be more easily incorporated in a single instrument, so that several of the same or different tests as well as reference tests, can be carried out simultaneously.

Electrochemical rather than photometric, detection on a strip also allows the use of simple materials such as filter paper without photo-absorbency constraints imposed by  
25 photometric measurements such as reflectance photometry. For example, it was found that in the detection of total cholesterol on a long strip it is important that its compositional characteristics including particle retention size do not have any unwanted separation effects on the analyte. It was found that standard glass fibre papers, which have advantageous photometric properties were not suitable because of this retention effect on cholesterol, and  
30 non-glass fibre papers with larger retention size, were found to be more suitable.

Electrochemical detection does not suffer from sample chromogenic or turbidity interferences associated with photometric detection methods.

- Another feature of some arrangements allows the transfer of sample, analyte, intermediate or end product from a sample receptacle such as a sample well, to the receiving zone,
- 5 allowing the analyte to undergo one or more separate steps before the electrochemically detectable en-product is produced. This includes pre-treatment reactions such as antibody-antigen binding in both heterogeneous and homogeneous immunoassays which can make use of the more favourable solution kinetics rather than solid phase kinetics reactions. It also includes DNA probe tests which would require a number of pre-treatment steps, such
- 10 as sample extraction, polymerase chain reaction, denaturing and hybridisation etc. before being transferred to the receiving and detection zone of the device. It also includes sample dilution; or pre-treatment with detergent etc. for example. It was also found that it was necessary to add the supporting electrolyte KCl to a separate strip from certain enzyme reagents such as those used in cholesterol determination to avoid loss of enzyme activity
- 15 during the drying process. The KCl can also be included in the sample well, in which case the reference electrode could be placed in the well, or anywhere along the feeder strip if desired. The carrying of sample along a fibrous strip to the reaction zone also oxygenates the sample which is useful where non-capillary sized samples are used in analyses involving glucose and cholesterol oxidase and other oxygen-dependent reactions.
- 20 The use of a fibrous matrix provides a means of carrying and distributing a single sample to several different reagent carriers and electrodes in linear or branched arrangements allowing for example, sample flow through a fibrous matrix to be increased, or multiple detections of analytes, calibrators and control samples.
- In contrast to photometric measurement, the detector, i.e. the electrode, must make contact
- 25 with the reagent strip since the end product is detected by an electrochemical reaction due to the applied potential at the electrode. In order to obtain reproducible results, it was found that it is necessary to take steps to ensure that the contact between the fibrous matrix and the electrode is also reproducible. This is achieved in one arrangement by applying an even and reproducible pressure to the electrode and strip and providing a firmly held base. The
- 30 method of applying this pressure is also important for reproducibility, in that it must be

applied in an instantaneous and rapid manner, such as a spring clamp rather than manually and slowly, as for example by using a screw action micrometer set to a particular thickness. Other ways of achieving reproducible contact might include reproducibly laminating or screen printing the electrode directly onto the surface of the strip, for example.

- 5 It was also found that when pressure is applied to the fibrous matrix it was necessary to prevent the squeezed liquid from leaking outside of the fibrous matrix and electrodes by an appropriate means such as by the use of an absorbent paper with sufficient absorbency to soak up all of this liquid. Since it was considered advantageous to allow the formation of a broad reaction zone without having to use immobilisation techniques either in the strip or  
10 on the electrode, it was found that this absorbent paper could also be used as a reagent carrier.

The order of placement of the absorbent strips and electrodes onto the fibrous matrix was also found to be important for reproducibility, in that it was found advantageous to first place the feeder strip onto the lower reagent strip, and to press down lightly to allow even  
15 contact between the soaked feeder strip and the lower reagent strip. The upper electrode and reagent carrier was then pressed down onto the feeder strip and reagent carrier and electrode below. Placing all three strips together simultaneously gave irreproducible results.

In contrast to photometric methods, when using a diluted sample, it is also necessary to  
20 incorporate a suitable supporting electrolyte such as potassium chloride (KCl) into the reagent strips. It was found that it was necessary to add the KCl to a separate strip from certain enzyme reagents such as those used in cholesterol determination to avoid loss of enzyme activity during the drying process. Alternatively KCl could be included in the sample well, but this may require adjusting the concentration of precipitation reagent due  
25 to the effect of higher electrolyte concentration. In this latter alternative, the reference electrode could be placed in the well, or anywhere along the feeder strip if desired.

According to an arrangement of the invention, sample is carried along a fibrous carrier matrix, preferably in the form of a strip, herein termed "feeder strips", connected by one or more fibrous matrix filters if desired. The particle retention size of the feeder strip is  
30 preferably large enough so as not to have any unwanted separation effects on the analyte,

whilst the particle retention size of the fibrous matrix filter material is small enough to retain the unwanted precipitate. For example, in the detection of cholesterol, the current obtained was lower than that of the equivalent concentration of the end product potassium ferricyanide when papers of low pore size were used as the feeder strip. It is thought that  
5 this may have been due to retention of a fraction of the cholesterol by the small pores, as expected current measurements were obtained when papers of larger pore size were used.

Some or all of the reagents necessary for the detection of the target analyte may be impregnated or immobilised into one or more reagent carriers attached to the electrode.

One or more membranes, may be applied to the feeder strip, reagent carriers or directly to  
10 the electrodes as a further means of analyte separation or as a means of excluding unwanted interferences. Alternatively the electrode material may be screen printed directly onto the reagent carrier. The reagent carriers may be made of suitable porous and preferably absorbent material such as for example, chromatography grade paper. Some or all of the reagents necessary for the detection of the target analyte may also be impregnated  
15 or immobilised into the feeder strip itself, or incorporated into the electrode material, such as by spin coating or by a screen printing process.

The reagent includes if necessary, an electrochemical mediator capable of taking part in electron transfer between the analyte, enzyme, or analyte reaction product, and the electrode. In the preferred arrangement, the mediator is preferably water soluble and could  
20 include for example, ruthenium, ferricyanide or ferrocyanide compounds such as potassium ferrocyanide for example. In the case of steady state amperometry, the mediator requirement is double the stoichiometric requirement of the maximum analyte concentration. This is so as to allow sufficient mediator for the analyte reaction sequence and subsequent redox reaction at the working electrode, as well as to allow an opposite  
25 redox reaction to take place at the auxiliary electrode. The reagent could also include if necessary an ion-exchange resin if desired, impregnated or immobilised into one or more reagent carriers attached to the electrode or incorporated into the feeder strip or incorporated into the electrode material, such as by spin coating or by a screen printing process for example.

In an arrangement of the invention for cholesterol measurement, a plasma or serum dilution of one in twenty was found to be optimal for the sensitivity of the detection system used. When this reaction is carried out in solution, and subsequently added to the feeder strip and detected immediately, bubbling air into the reaction solution was found to be  
5 necessary. This was found not to be necessary when the sample was applied to the feeder strip and the reaction allowed to take place in the reagent carriers.

In one arrangement, reagents are vacuum dried onto strips of chromatography grade paper, at concentrations allowing for the total required concentration after reconstitution with sample. The feeder strip is preferably held apart from the reagent carriers to allow the  
10 feeder strip to become saturated by the sample before coming into contact with the reagent. This prevents the sample from washing reagent to the end of the reagent carrier presenting a larger and more uniform reaction zone to the electrode surface. It is also preferable to apply pressure to the electrodes to ensure good contact with the fibrous matrix. It is also preferable that the capacity of the fibrous reagent carriers is sufficient to  
15 absorb the liquid squeezed from the feeder strip when this pressure is applied. The reagent carriers are preferably placed so as to extend slightly over and beyond each electrode to allow positioning of the reference electrode, attachment to a spacer and also to compensate for any washing forward of reagents on the carriers, when contacted by the soaked feeder strip. The electrodes and reagent carriers are preferably attached to a firm support to ensure  
20 even pressure.

The feeder strip and reagent carriers can be held apart from each other by means of a device amenable to electromechanical automation.

The working and auxiliary electrodes are made of preferably screen printed graphite ink, or graphite foil, or other material suitable for use as working and auxiliary electrodes. The  
25 reference electrode is made of preferably silver/ silver chloride screen printed ink, or other material suitable for use as reference electrodes.

In amperometric detection, the reaction product is measured by poisoning the working electrode at a potential, relative to the reference electrode, which reduces or oxidises the electrochemically detectable analyte reaction end product. The oxidation or reduction at the  
30 working electrode produces a current proportional to the concentration of the end product.

and therefore the amount of analyte present. At the other, auxiliary electrode, an equal and opposite reaction is allowed to take place. Placing the auxiliary and working electrodes adjacent and close to each other has the effect of setting up a continuous cycle so that the resulting current settles to a steady state, so that the time at which the current is read is not critical.

In the preferred arrangement, the working and auxiliary electrodes are placed on opposite sides of the feeder strip with the reference electrode on either of the sides. In another arrangement, an interdigital array or similar planar electrode arrangement comprising of working and auxiliary electrodes for obtaining a steady state current, made of screen printed graphite or other suitable material, can be placed on one side and the reference electrode on either side, of the fibrous carrier matrix. In a variation of this configuration, the working and auxiliary electrodes can be placed on the same side of the feeder strip, in a screen printed interdigital array to give the same steady state effect, with the reference electrode placed on the opposite side of the feeder strip.

The sample may be applied to the feeder strip directly, but preferably it is added to a sample well. The well may contain a fixed volume of sample diluent, such as deionised water or buffer as for example in total cholesterol measurement. Alternatively, the well may contain either in freeze-dried form or in solution, any or all of the reagents required to carry out the electrochemical measurement. Alternatively the sample well can contain a precipitation reagent, such as for example, any standard reagent to precipitate non-HDL sub-fractions, LDL-cholesterol, or latex particles coated with antibody to non-LDL cholesterol.

The precipitation reagent may also be one or more different receptors or ligands such as antibody, antigen, DNA, RNA etc. preferably coated onto latex or other particles with one or more different labels if desired.

The label may be electrochemically detectable such as for example, a DNA probe linked to a ferrocene derivative (Anal. Biochem. (1994) 218: 436-443), or the label may be an enzyme such as for example, alkaline phosphatase, which can be used with substrates such as 4-aminophenylphosphate, 4-nitrophenylphosphate, or phenylphosphate, to give on hydrolysis 4-aminophenol, 4-nitrophenol (Anal. Biochem. (1991) 192: 92-5) and phenol

(Clin. Chem. (1985) 31/9: 1546-9). Alternatively these substrates or any other substance which could be measured by electrochemical means such as amperometric or other voltammetric or coulometric methods, could be used as label. The reagent for detecting the labeled receptor/ligand could also be an enzyme substrate such as those mentioned above, which on hydrolysis gives an electrochemical substance, or the reagent could be an enzyme such as alkaline phosphatase, which reacts with a substrate to give a substance capable of being measured by electrochemical means. The reagent may also include if desired an enzyme amplification sequence containing for example, NADP and alcohol dehydrogenase, diaphorase and an electrochemical mediator such as potassium ferricyanide. The reagent may also include an ion-exchange resin if desired, impregnated or immobilised into one or more reagent carriers attached to the electrode or incorporated into the feeder strip or incorporated into the electrode material.

The sample may comprise any liquid or liquid extract of a clinical sample, such as whole blood, plasma or serum, urine, sputum etc. The sample well may be used in conjunction with a device for separating plasma from whole blood, for example, a centrifuge device, with integrated sample delivery device, such as a disc, or a sample dispenser, or other device for this purpose. The sample well may also be used in conjunction with a device for transferring sample through several chambers or compartments to allow different sample pre-treatment reactions, such as sample extraction, etc. to take place.

For the measurement of cholesterol or the sub-fractions of cholesterol, detergent is added preferably to the feeder strip as well as the reagent strips, to minimise the reaction time. A suitable supporting electrolyte such as potassium chloride (KCl) can be incorporated into the reagent strips. The KCl is added preferably to a separate strip from the enzyme reagents to avoid loss of enzyme activity during the drying process. Alternatively KCl could be included in the sample well, but this may require adjusting the concentration of precipitation reagent due to the effect of higher electrolyte concentration. In this latter alternative, the reference electrode could be placed in the well, or anywhere along the feeder strip if desired.

In the preferred arrangement, after sample has reached the end of the last feeder strip, it is first applied to the lower reagent strip and working electrode and pressed down lightly and

uniformly. The auxiliary electrode and reagent strip is then pressed down firmly by applying a reproducible and even pressure. This is achieved for example by means of a spring-action clamp, or similar device, or alternatively by electromechanical means incorporated in the instrument. The mechanical pressing device and the solid support for the configuration are preferably held firmly to avoid irreproducibility brought about by changes in the pressure applied.

The temperature of the reaction zone can be controlled if desired by means of a temperature controller and a thermistor held close to the reaction zone. After sufficient reaction time, the concentration of the analyte can be determined by electrochemical means including voltammetric or coulometric methods.

A reference standard made up to a known final concentration could be added to the sample well and used as a calibrator with the sample together with a blank reagent strip as required; e.g. in the case of cholesterol measurement, using a reagent strip with all the reagents for cholesterol detection except cholesterol oxidase. Alternatively a reference standard could be dried onto one of the strips, or incorporated into one of the electrodes, to a known reconstituted concentration, for calibration purposes. This might also be incorporated into a further arrangement in which a single feeder strip carries sample to more than one separate reagent carrier or set of reagent carriers, so as to allow the measurement of for example, a sample and calibrator standard in a single test.

The device can be used for various clinical analyses preferably but not necessarily including, those involving a separation step, such as the cholesterol sub-fractions, and various immunoassay and DNA probes. For example, it is well known that latex particles with adsorbed or covalently bound antigen or polyclonal antibody can be used to effect agglutination between sample antibody or antigen, respectively. The agglutination can be determined qualitatively by visual inspection, or quantitatively by instrumental methods which have been recently developed using various methods such as photometric particle counting, or turbidometry.

The device can be used to carry out quantitative immunoassay determinations based on latex particle agglutination. For example, latex particles with adsorbed or covalently bound antigen or polyclonal antibody labeled with alkaline phosphatase can be used to effect

agglutination with the target antibody or antigen respectively in the sample well. The agglutinated latex particles can be retained by a suitable fibrous matrix connected to the feeder strip. The electrodes and reagent can be brought into contact with the retained particles directly or preferably with the remaining uncomplexed labeled antibody or antigen latex particles after filtration has been effected. In the latter case, the amount of analyte present is determined by subtraction from a control current obtained for example by carrying out the same reaction using a non-specific labeled polyclonal antibody or antigen with the sample.

As a further example of a quantitative immunoassay determination using the described device, monoclonal antibody labeled with for example, alkaline phosphatase can be used to effect complex formation with target antigen in the sample well. The antigen-antibody complex can be carried along the feeder strip and captured by an immobilised polyclonal antibody, and brought into contact with the electrodes and reagent containing 4-aminophenylphosphate, for example. The remaining uncomplexed labeled antibody or antigen can be carried to the end of the feeder strip by means of an absorbent pad attached to the end of the feeder strip if desired.

The device can also be used to carry out quantitative DNA and RNA determinations using DNA probes such as in the identification of bacterial ribosomal RNA using various known methods. It is known for example that single stranded DNA probes can be labeled with enzymes such as alkaline phosphatase, and that specific polyclonal or monoclonal antibodies have been used to effect complex formation with target RNA:DNA hybrids (Anal. Biochem. (1988) 169:1-25).

In one example using these methods with the device of the invention, single stranded DNA probe labeled with for example alkaline phosphatase can be allowed to hybridise with the target RNA in the sample well. Latex particles with adsorbed or covalently bound polyclonal antibody can be added to effect agglutination of the hybridised target RNA. After allowing the reaction to go to completion, the agglutinated latex particles can be retained by a suitable fibrous matrix connected to the feeder strip. The electrodes and reagent can be brought into contact with the retained particles directly or preferably with the remaining non-hybridised labeled DNA probe after filtration has been effected. In the

latter case, the amount of RNA present can be determined by subtraction from a control current obtained for example by carrying out the same reaction using a non-complementary labeled DNA probe with the sample.

The above example can also be carried out for example by using labeled polyclonal antibody and DNA probe coated onto small diameter latex particles, which are known to promote hybridisation rates as fast as those obtained in solution (Nucleic Acids Res. (1987) 15: 2911-2926). The latex DNA:RNA hybrid antibody complex is filtered on the feeder strip and the remaining uncomplexed labeled antibody can be brought into contact with the electrodes and reagent.

- 10 Alternatively, hybrid formed in the sample well, comprising labeled DNA and target RNA can be captured by immobilised DNA:RNA antibody, on the feeder strip and brought into contact with the reagent carriers and electrodes. The remaining uncomplexed DNA can be carried in the sample to the end of the feeder strip, by means of an absorbent pad of fixed absorbency attached to the end of the feeder strip if desired.
- 15 The immobilised ligand or receptors can be used to effect ligand-receptor capture such as antibody-antigen or DNA-DNA hybrids, DNA-RNA hybrids, or antibody-DNA-RNA hybrids for example.

- The device can be used to carry out determinations for cholesterol and other clinical chemistry analytes by various electrochemical methods. The detection of cholesterol, and other analytes such as glucose and lactate for example can be based on the amperometric reduction of a mediator oxidised by hydrogen peroxide catalysed by peroxidase enzyme. Other examples include those based on the amperometric oxidation of mediators such as potassium ferrocyanide reduced by NADPH/NADH catalysed by lipoamide dehydrogenase enzyme (diaphorase). Such analytes include for example, alanine
- 20 aminotransferase (ALT/GPT), alcohol, amylase, aspartate aminotransferase (AST/GOT), creatine kinase and lactate dehydrogenase (LDH). Other tests including  $\gamma$ -glutamyl transferase ( $\gamma$ -GT) and bilirubin can be carried out using amperometric detection of azo-dye formation for example.
- 25

Whilst the invention is described herein principally in relation to its use in medical, clinical diagnostic use, it will be appreciated that it is equally applicable to veterinary clinical use. Furthermore, it has useful applications in industrial and experimental laboratory practice.

5 The invention will now be described more particularly with reference to the accompanying drawings which show, by way of example only, an embodiment of an electrochemical diagnostic device according to the invention.

In the figures:

Figure 1 is a side view of the device prior to use;

Figure 2 is a perspective view similar to Figure 1;

10 Figure 3 is a perspective view similar to Figure 2, including a slider assembly;

Figures 4(a) to (d) are plan, plan, side and perspective views respectively of components of the slider assembly of Figure 3;

Figure 5 is a calibration curve for total cholesterol; and

Figure 6 is a calibration curve for HCl cholesterol.

15 Referring initially to Figures 1 and 2, the device includes a rectangular piece of glass 22, (2.5cm x 4.2 cm). This rests on a metal stand 23 and is secured by a double sided tape. A further glass platform 21 (0.5cmx 2.3cm) is placed at the end of the rectangular glass base.

A rectangular screen printed graphite working electrode 16, measuring 3cm x 0.5cm is stuck onto the glass platform 21 so as to allow 1.5cm of the electrode to contact the  
20 platform, and 1.5cm to protrude at the end for crocodile clip connection 17 to a potentiostat (not shown).

A rectangular screen printed reference electrode 19, measuring 3.0cm x 0.5cm is positioned laterally using double-sided tape, so that only 0.5cm of its length contacts the beginning of the platform. The reference electrode is separated from the working electrode by a  
25 polyester spacer 18 measuring 0.3cm x 0.5cm, attached to the glass platform by means of double sided tape, and also with double-sided tape on its upper surface.

To the double-sided tape on the surface of the polyester spacer is attached a reagent strip, for example an enzyme reagent strip 15 so as to cover the entire length of the reference electrode, spacer and working electrode.

On the base of the metal stand 23 is attached a platinum resistance thermometer (Radionics, Dublin), connected to an automatic temperature controller (Omron, Automated Technical Controls, Dublin) connected to a coil heater via a solid state relay (not shown).

One end (0.5 cm in length) of the first feeder strip 2 measuring 2.3cm x 0.5cm is attached to the beginning of the glass base plate 22. Three spacer discs 3-5 made of Ahlstrom #939 discs 0.6 cm diameter are placed on top of this end. The other end of the feeder strip protrudes from the beginning of the base plate, and is poised over the sample well 1.

To a glass strip 9 measuring 2.3cm x 0.5cm is attached by double-sided tape, a screen printed graphite auxiliary electrode 10, so that 1.5 cm of its length contacts the end of the glass strip, and 1.5cm protrudes, to allow attachment of the connection 11 to the potentiostat. To the remaining part of the glass strip is attached a polyester spacer 8, measuring 0.8 cm x 0.5 cm, to which is attached a reagent strip 12 by means of double sided tape. The reagent strip may be a KCl strip for cholesterol testing.

The second feeder strip 13 is held onto the plastic support 6 by means of a polyester backing 7 to overlap the spacer discs at one end, and is held apart from and poised, over the enzyme strip 15, by means of a plastic holder 14 or, as an alternative, the lower part of the slider device described below with reference to Figures 3 and 4.

A slide action assembly 32 as shown in Figures 3 and 4 can be used to hold the feeder strip 13 apart from the reagent carriers. The lower part 24 of the assembly is placed so that its three arms 33 lie between the reagent strip 15 below and the feeder strip 13 above. The lower part 24 of the assembly is separated from the upper part 25 by a spacer 30, measuring about 2.3mm thick, and 1.1 cm x 2.3 cm wide.

The upper part of the device 25 comprises a rectangular polyester support, 4.5 cm x 2.3 cm as shown in Figure 3 (b). On the underside of this, approximately 7mm in from the end is attached a strip of thick gauze (mesh 10T) (not shown) measuring 2.3 x 0.5cm. The purpose of this is to prevent the soaked feeder strip from sticking to the underside of the polyester. On the upper side of the polyester and directly over this gauze is attached a small plastic support 2.3cm x 0.5cm. The lower and upper parts of the device, together with the spacer form the slider 31 which rides in the slide casing 29 by means of the guide wings 24a on the lower part of the slider which move in recesses 29a of the slide casing 29.

A parallel jaw holder 20 a and b (Figure 1) (Radionics, Dublin Ireland) is held in place so that the lower jaw gripped the underside of the metal stand 23. The top electrode, reagent carrier and glass strip 9 is attached either directly to the upper parallel jaw or to the end of the polyester support 25 of the slider. The two jaws are held open until contact is required.

- 5 The operation of the device will now be described using an assay for total cholesterol as an example.

**Buffer:**

1 litre of double strength phosphate buffer was prepared by adding:

25g  $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$

- 10 10.62g  $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$

to 1 litre of deionised water.

**Reagent 1:**

To 44.75mls of buffer was added:

1.889g Triton X-100,

- 15 and made up to 50 mls with deionised water. To this was added:

0.4629g cholic acid.

**Reagent 2: (KCl reagent strip)**

To 10 mls reagent 1 was added:

0.3997g KCl.

- 20 **Reagent 3 (ferrocyanide mediator):**

To 10 mls reagent 1 was added:

0.2831g  $\text{K}_4\text{Fe}(\text{CN})_6$ .

**Reagent 4:**

To 25 mls of reagent 1 was added:

- 25 horse radish peroxidase (315U/mg): 0.0048g

pancreatic cholesterol esterase (12.5U/mg): 0.0192g

microbial cholesterol esterase (10.8U/mg): 0.0222g.

**Reagent 5: (Enzyme A)**

To 12.5 mls of reagent 4 was added:

cholesterol oxidase (10.7U/mg), 0.0114g.

**Reagent 6: (Enzyme B)**

To 12.5 mls of reagent 4 was added:

0.0057g of pancreatic cholesterol esterase

5 0.0057g of microbial cholesterol esterase.

**Reagent 7: (Triton for Feeder)**

To 25mls of deionised water was added:

1g of Triton X-100.

- 10 Reagent strips were prepared by cutting Whatman 4Chr sheets (Whatman Paper Ltd., Maidstone, Kent, UK) into strips measuring 2.3x0.5cm. Feeder strips were prepared by cutting Ahlstrom sheets of grade either #939, (stated particle retention size 55 $\mu$ m), or #1281 (stated mean pore size 29.7  $\mu$ m) (Ahlstrom Filtration Inc., Holly Springs, PA USA) into strips measuring 4.2x0.5cm.
- 15 In a darkened room, two parts of reagents 2 (KCl), 5 (Enzyme A) and 6 (Enzyme B) were added to one part of reagent 3 (ferrocyanide mediator). Reagents 2,5 and 6 were then applied to the 4Chr reagent strips in 26.8  $\mu$ l aliquots. Reagent 7 (Triton) was added to the feeder strips in 42.2  $\mu$ l aliquots. The strips were vacuum dried using a high vacuum pump and nitrogen trap, for 90 minutes in the dark.
- 20 Graphite electrodes were prepared using Electrodag 423 SS graphite ink (Acheson Colloids, Plymouth UK) screen printed in strips of 3cm x 25cm onto polyester backing using #70 T polymon mesh, and jet dried for 2 minutes at 120°C. Silver /silver chloride strips for the reference electrodes were prepared using Electrodag DB 2268 (Acheson Colloids) using the same method.
- 25 Interdigital array electrodes were prepared using the 423 SS graphite ink, and Electrodag 970 SS dielectric ink (Acheson Colloids) as insulator.

The measuring apparatus consisted of a Thompson Ministat potentiostat (H.B. Thompson & Associates, Newcastle-upon-Tyne UK), a Lloyds flatbed chart recorder, and a variable resistor (JJ Lloyd Instruments, Southampton, UK).

The potentiostat was set to -200 mV and its output connected to the chart recorder via the variable resistor set at 8 kilo-ohms, the sensitivity of the chart recorder being set to 20 mV/10cm to give a scale of 0.25 $\mu$ A/cm. The reaction temperature was set to 37°C.

The sample well contained 450 $\mu$ l of phosphate buffer, pH 7.4. To this was added 25 $\mu$ l of serum/plasma sample containing a known concentration of cholesterol measured photometrically, and 25 $\mu$ l of deionised water (with enzyme A strip), or potassium ferricyanide ( $K_3Fe(CN)_6$ ) solution containing double the known cholesterol concentration (with enzyme B strip). After mixing, the end of the first feeder strip was dipped into the sample well, allowing it to flow to the end of the second feeder strip. After 2 minutes, the feeder strip was placed down onto the lower reagent strip, and pressed down lightly with tweezers to allow even contact between the soaked feeder strip and the lower reagent strip. The upper jaw 20(a) of the parallel jaw holder with auxiliary electrode 10, KCl reagent carrier 12 and glass strip 9 was then released and allowed to press down onto the feeder strip 13 and lower reagent carrier 15 and working electrode 16.

Alternatively, if the slider assembly was used, the slider 31 was moved from its position 1 to position 2 (see Figure 3(d)) so that the arms 33 were withdrawn from between the feeder 13 and the lower reagent carrier 15, and the gauze strip came to lie over the feeder strip. The feeder strip was manually prevented from being displaced using tweezers. This might also be achieved by, for example, the use of one or more small guides. After lightly pressing the gauze down onto the feeder strip, the slider was moved to position 3 to align the auxiliary electrode 10 and reagent carrier 12 with the feeder strip 13 (see Figure 3(d)). This was pressed down onto the feeder strip 13 by means of the parallel jaws (20).

The reaction was allowed to proceed for about 7.5 minutes, then the potential was applied. The resulting current was recorded and the steady state current measured after 3.5 minutes.

The currents obtained using enzyme A and B strips were compared.

In a further experiment, a series of serum/plasma samples were measured using this configuration, and the resulting currents plotted against concentration to produce a calibration curve.

The results are shown in Figure 5. In the experiments, a ferricyanide standard was used. The Figure represents a calibration curve for total cholesterol.

The following is an example of the use of the device for the measurement of HDL cholesterol.

- 5 For the measurement of HDL cholesterol, HDL precipitant (Boehringer Mannheim GmbH Germany, cat no. 543004) containing phosphotungstic acid (PTA) 0.55mM and magnesium chloride 25mM was used. The middle of the three spacer discs (5) was replaced by a GD-120 or GB-140 glass fiber filter disc, diameter 0.6cm (stated pore size, 2.7 $\mu$ m and 1  $\mu$ m respectively) (Micro Filtration Systems, Dublin, California, USA).
- 10 To 560 $\mu$ l of PTA was added 140 $\mu$ l of water and 280 $\mu$ l of sample. After waiting for a minimum of 3 minutes, 450 $\mu$ l of this was centrifuged for 10 minutes at 10,000 rpm and kept for use in the device. The supernatant or the precipitate suspension was added to the sample well to which the feeder strip was then introduced. The reaction was performed as with total cholesterol. The currents were compared for three pairs of a single HDL
- 15 cholesterol value obtained by filtration or by centrifuge. A calibration curve was obtained for a series of serum/plasma samples of known HDL cholesterol concentration and is shown in Figure 6.

- The currents obtained for total cholesterol with ferricyanide standard, and for spun and filtered HDL cholesterol samples compare well. The calibration curves for total and HDL
- 20 cholesterol are linear throughout the range of values likely to be encountered clinically.

It will be understood that the invention is not limited to the specific details described herein, which are given by way of example only, and that various modifications and alterations are possible within the scope of the appended claims.

CLAIMS:

1. A device for conducting a diagnostic test in clinical, veterinary, industrial or laboratory applications on a sample comprising an analyte which is capable of reacting with a reagent  
5 in a reaction or reaction sequence to produce an electrochemically detectable end-product, optionally via an intermediate-product, the device comprising an elongate fibrous carrier matrix (2,13) connectable with a reference (19) and an auxiliary (10) electrode having a receiving zone (1) for receiving the sample, analyte intermediate- or end-product in a liquid medium and a detection zone to which the sample, analyte, intermediate- or end-product is  
10 transferred by capillary action, and a working electrode (16) contactable in use with the detection zone, for electrochemically detecting the end-product by amperometric, coulometric or other voltammetric methods, when electrical conductivity between the electrodes through the liquid medium and wet matrix is established, the device also including a reagent for reacting with the sample, analyte or the intermediate-product to  
15 produce the end-product in the case that the sample, analyte or the intermediate-product is received on the receiving zone of the fibrous carrier matrix.
2. A device according to claim 1, comprising at least two connectable fibrous carrier matrices (2,13) of the same or different composition, in linear or branched arrangements, at  
20 least one of the fibrous matrices having a detection zone contactable with a working electrode.
3. A device according to claim 2, in which the at least two fibrous carrier matrices are in branched arrangement to enable multiple detections of analytes, calibrators and control  
25 samples on the branched fibrous matrices.
4. A device according to any preceding claim, in which the fibrous matrix is selected to effect one or more separation steps in which an analyte, or an interfering analyte, or an intermediate or end product is retained during transit through the fibrous matrix.

5. A device according to any proceeding claim for conducting a diagnostic test on a sample comprising more than one analyte, intermediate- or end-product which can be received on the receiving zone.

5

6. A device according to any preceding claim, in which the intermediate or end product received on the receiving zone or transferred to the detection zone comprises a labeled ligand-receptor complex.

10 7. A device according to claim 6, in which the labeled ligand-receptor complex comprises a labeled antibody-antigen, or a labeled DNA-DNA or DNA-RNA complex, or an end product comprising a ligand-receptor complex with an electrochemically detectable label, or an intermediate product comprising a ligand-receptor complex with a label which is capable of reacting with a reagent in a reaction or reaction sequence to produce an  
15 electrochemically detectable end product.

8. A device according to claim 6 or claim 7, in which two or more different ligand-receptor complexes having the same label are detectable at different detection zones.

20 9. A device according to claim 6 or claim 7, in which two or more different ligand-receptor complexes having different electrochemically detectable labels are used and the device includes means for applying different potentials in the same detection zone to detect the different labels.

25 10. A device according to any preceding claim, in which the detection zone includes a zone on the fibrous matrix which in use contacts a piece or a stack of two or more pieces of porous or absorbent material (3-5) to which the working, reference or auxiliary electrodes are contactable.

11. A device according to any preceding claim, in which the sample receiving zone is in contact with a sample holder (1) capable of holding the sample.
- 5 12. A device according to claim 11, in which the sample holder comprises a hydrophobic gauze, or a receptacle or sample well, or a device capable of carrying out one or more sample pre-treatment processes.
- 10 13. A device according to any proceeding claim, in which a reagent, or a background reagent such as buffer, supporting electrolyte, or surfactant, necessary for producing or detecting the end product is carried in a reagent carrier contactable with the fibrous matrix, the reagent carrier comprising a sample well, or a fibrous carrier matrix, or an electrode by incorporation into the electrode material, or a piece of porous or absorbent material or a piece of membranous material contactable with the fibrous matrix or working, reference or  
15 auxiliary electrode.
- 20 14. A device according to claim 1, in which the electrodes are arranged so as to allow electrical conductivity through the liquid medium and wet matrix between a different auxiliary, reference or reference/auxiliary electrode and each of the one or more working electrodes, or between a common auxiliary, reference or reference/auxiliary electrode and two or more working electrodes, the reference, auxiliary or reference/auxiliary electrodes being connectable with the fibrous carrier matrix either directly or through one or more pieces of porous or absorbent material or through the sample liquid in a sample well.
- 25 15. A device according to claim 14, in which the working and auxiliary electrodes are made of screen printed graphite ink, or graphite foil, or other material suitable for use as working and auxiliary electrodes.

16. A device according to claim 14, in which the reference electrode is made of silver/silver chloride screen printed ink, or other material suitable for use as reference electrodes.

- 5 17. A method for conducting a clinical, veterinary, industrial or laboratory diagnostic test, comprising

reacting an analyte in a sample with a reagent in a reaction or reaction sequence to produce an electrochemically detectable end-product, optionally via an intermediate-product,

10

applying the sample, analyte, intermediate- or end-product in a liquid medium to a receiving zone of an elongate fibrous carrier matrix (2,13),

- 15 in the case of the receiving zone of the fibrous carrier matrix receiving the sample, analyte, or intermediate-product, reacting the sample, analyte or the intermediate product with a reagent included in the device to produce an electrochemically detectable end-product,

connecting the fibrous carrier matrix with a reference (19) and an auxiliary (10) electrode, and

20

transferring the sample, analyte, intermediate- or end-product by capillary action to a detection zone on the fibrous matrix, contacting the detection zone with a working electrode (16) and electrochemically detecting the end product by amperometric, coulometric or other voltammetric methods when electrical conductivity between the  
25 electrodes through the liquid medium and wet matrix is established.

18. A method according to claim 17, comprising providing at least two connectable fibrous carrier matrices of the same or different composition, in linear or branched arrangements, at least one of the fibrous matrices having a detection zone contactable with a working electrode.

5

19. A method according to claim 18, in which the at least two fibrous carrier matrices are in branched arrangement to enable multiple detections of analytes, calibrators and control samples on the branched fibrous matrices.

10 20. A method according to any of claims 17 to 19, in which the fibrous matrix is selected to effect one or more separation steps in which an analyte, or an interfering analyte, or an intermediate or end product is retained during transit through the fibrous matrix.

15 21. A method according to any of claims 17 to 20 for conducting a diagnostic test on a sample comprising more than one analyte, intermediate- or end-product which can be received on the receiving zone.

20 22. A method according to any of claims 17 to 21, in which the intermediate or end product received on the receiving zone or transferred to the detection zone comprises a labeled ligand-receptor complex.

25 23. A method according to claim 22, in which the labeled ligand-receptor complex comprises a labeled antibody-antigen, or a labeled DNA-DNA or DNA-RNA complex, or an end product comprising a ligand-receptor complex with an electrochemically detectable label, or an intermediate product comprising a ligand-receptor complex with a label which is capable of reacting with a reagent in a reaction or reaction sequence to produce an electrochemically detectable end product.

24. A method according to claim 22 or claim 23, in which two or more different ligand-receptor complexes having the same label are detected at different detection zones.

25. A method according to claim 22 or claim 23, in which two or more different ligand-receptor complexes having different electrochemically detectable labels are used and  
5 different potentials are applied in the same detection zone to detect the different labels.

26. A method according to any of claims 17 to 25, in which the detection zone includes a zone on the fibrous matrix which in use contacts a piece or a stack of two or more pieces of  
10 porous or absorbent material to which the working, reference or auxiliary electrodes are contactable.

27. A method according to any of claims 17 to 26, including contacting the sample receiving zone with a sample holder capable of holding the sample.

15

28. A method according to claim 27, in which the sample holder comprises a hydrophobic gauze, or a receptacle or sample well, or a device capable of carrying out one or more sample pre-treatment processes.

20 29. A method according to any of claims 17 to 28, including carrying a reagent, or a background reagent such as buffer, supporting electrolyte, or surfactant necessary for producing or detecting the end product in a reagent carrier which is in contact with the fibrous matrix, the reagent carrier comprising a sample well, or a fibrous carrier matrix, or an electrode by incorporation into the electrode material, or a piece of porous or absorbent  
25 material or a piece of membranous material contactable with the fibrous matrix or working, reference or auxiliary electrode.

30. A method according to claim 17, including arranging the electrodes so as to allow electrical conductivity through the liquid medium and wet matrix between a different auxiliary, reference or reference/auxiliary electrode and each of the one or more working electrodes, or between a common auxiliary, reference or reference/auxiliary electrode and  
5 two or more working electrodes, the reference, auxiliary or reference/auxiliary electrodes being connectable with the fibrous carrier matrix either directly or through one or more pieces of porous or absorbent material or through the sample liquid in a sample well.

31. A method according to claim 30, in which the working and auxiliary electrodes are  
10 made of screen printed graphite ink, or graphite foil, or other material suitable for use as working and auxiliary electrodes.

32. A method according to claim 30, in which the reference electrode is made of silver/silver chloride screen printed ink, or other material suitable for use as reference  
15 electrodes.

33. A kit for conducting a clinical, veterinary, industrial or laboratory diagnostic test, the kit comprising a device according to claim 1 together with the reagent or reagents necessary for performing the test.  
20

34. A kit according to claim 33, in which the test is a human or veterinary clinical chemistry test, or a homogeneous or heterogeneous ligand-receptor binding assay such as an immunoassay or DNA probe test.

25 35. A kit according to claim 34, in which the test measures blood total cholesterol and/or cholesterol sub-fraction levels.

36. A kit according to claim 34, in which the test measures one or more hormone levels.

37. A kit according to claim 34, in which the test measures one or more infections agents or one or more antibodies raised in the human or animal body against an infectious agent.

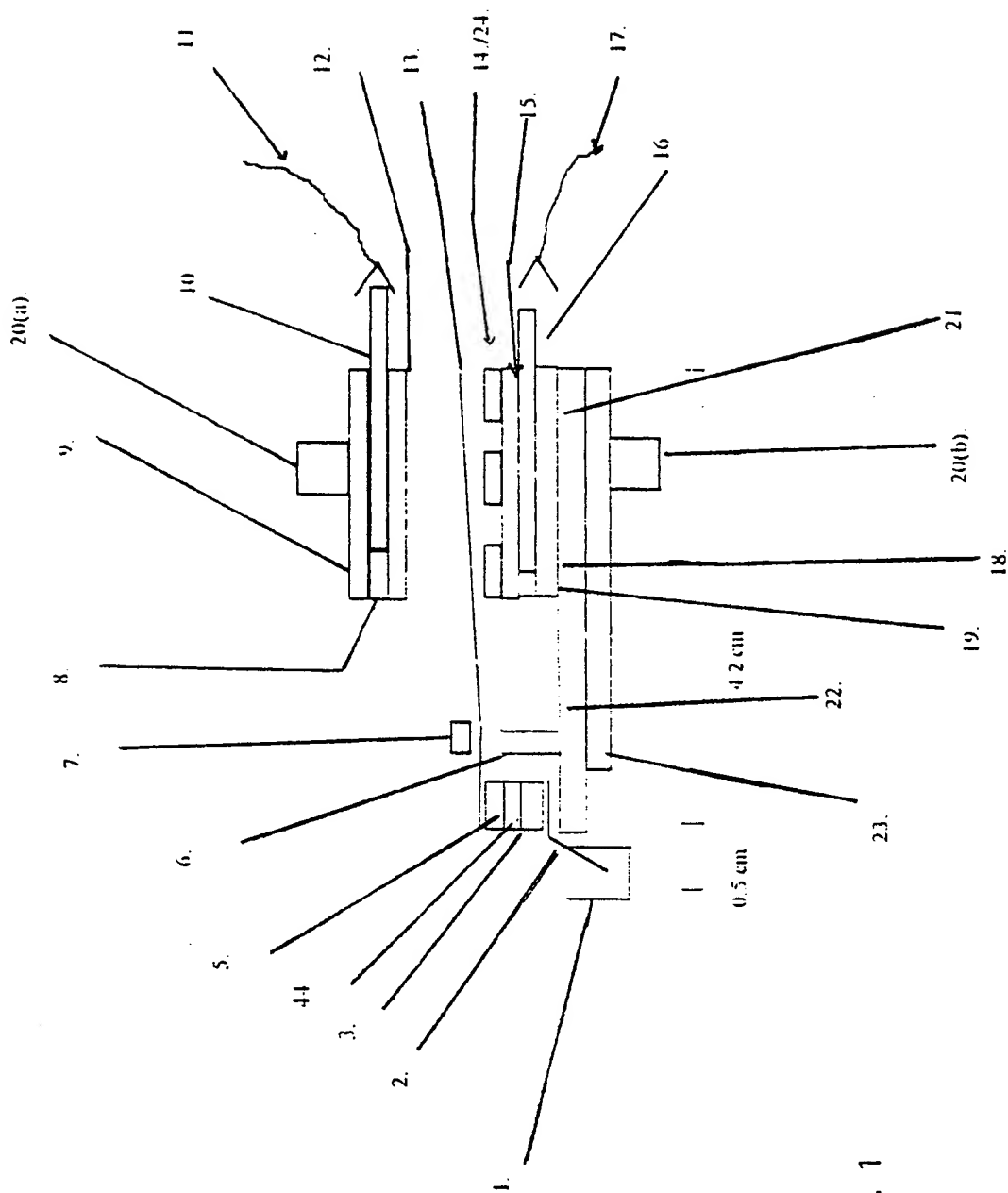
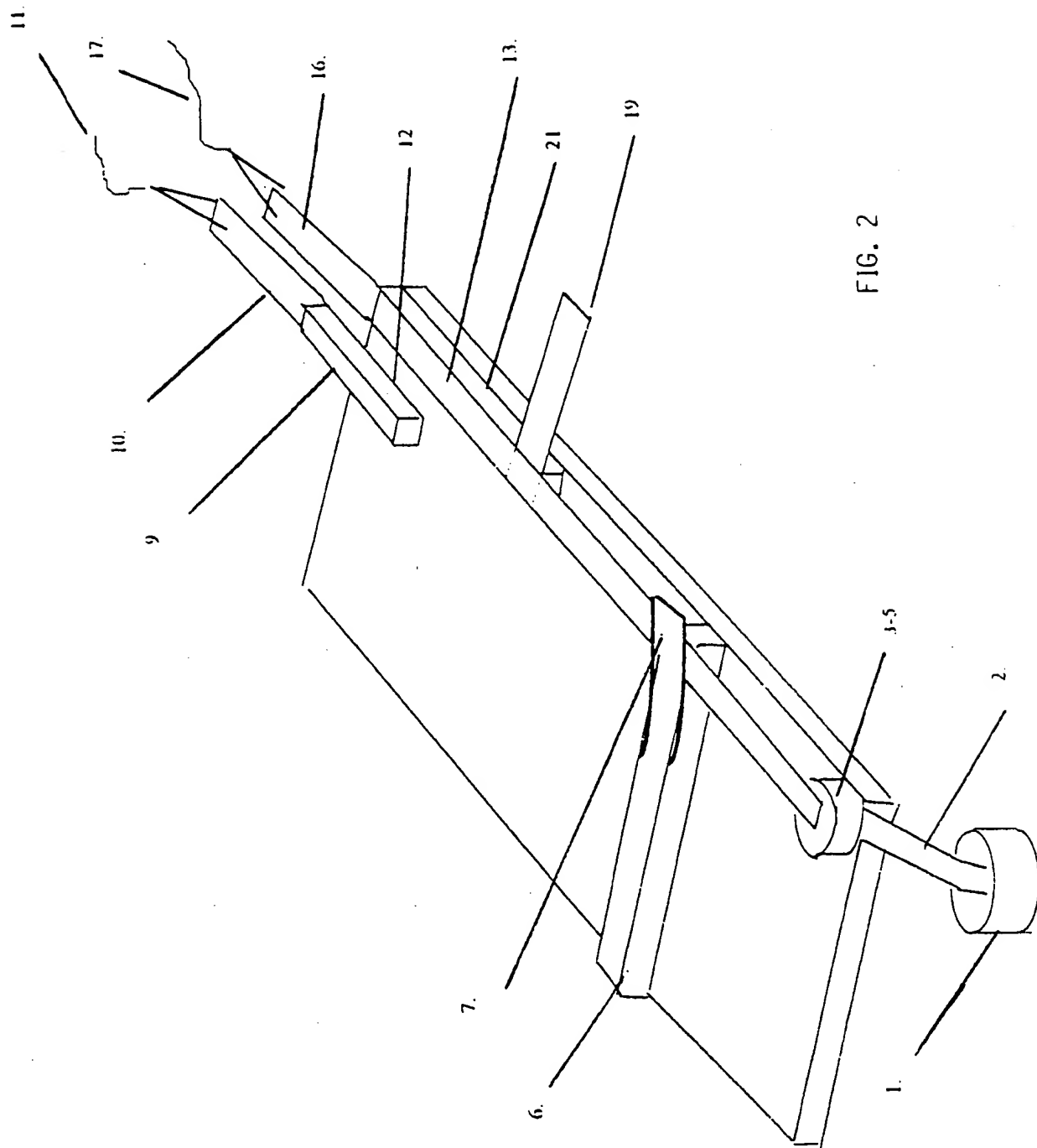
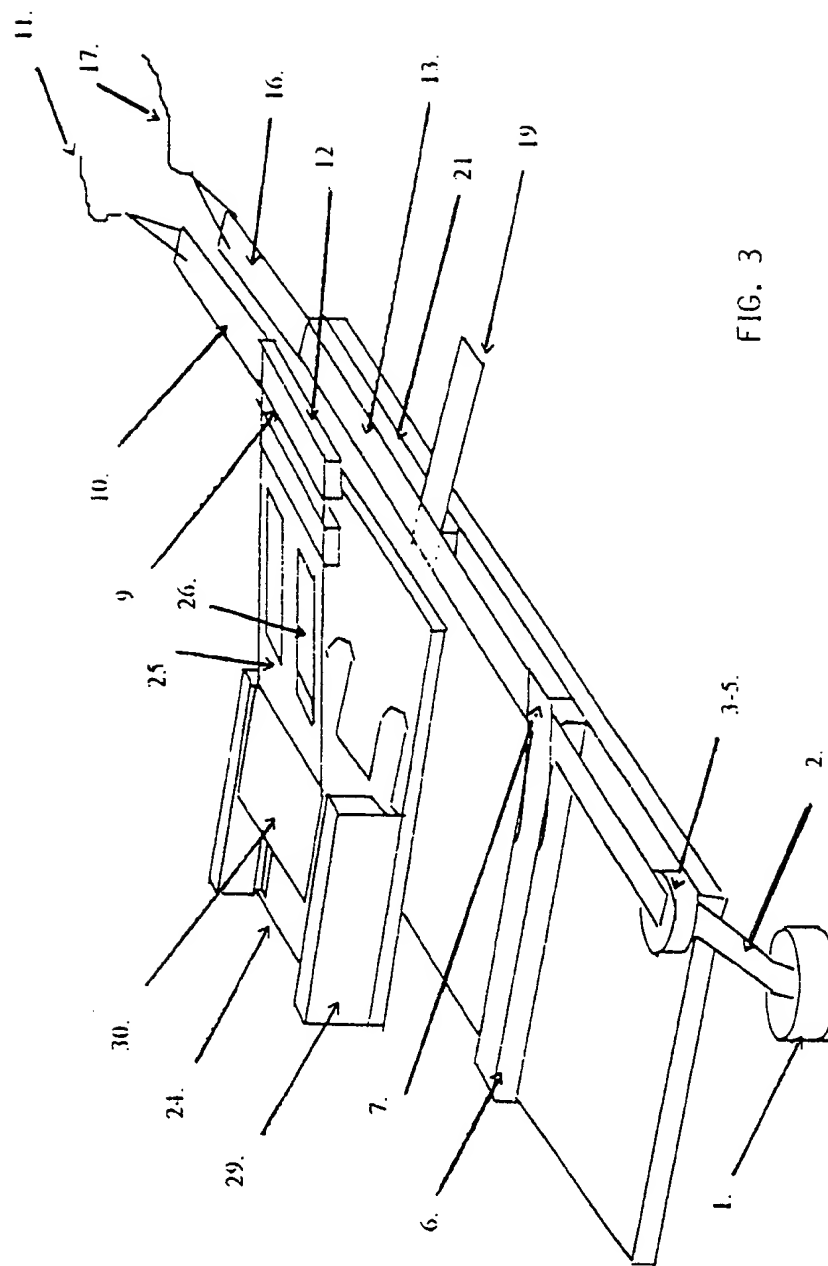


FIG. 1



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FIG. 4(a)

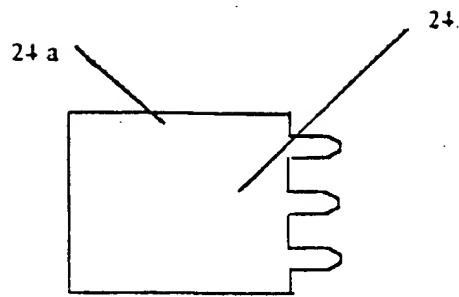


FIG. 4(b)

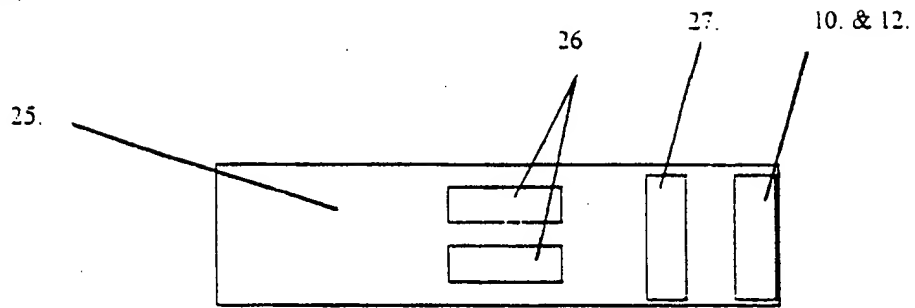


FIG. 4(c)

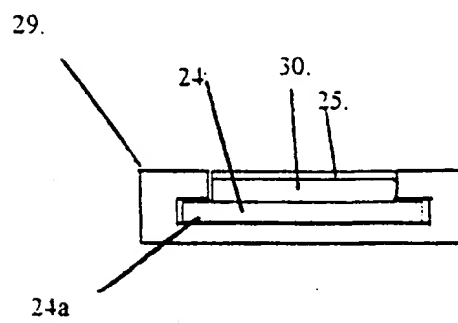
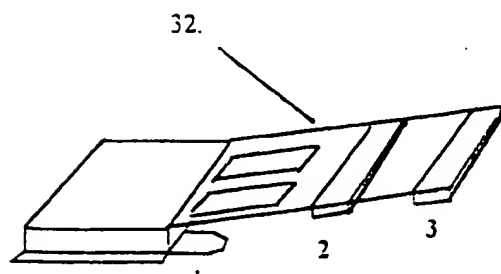
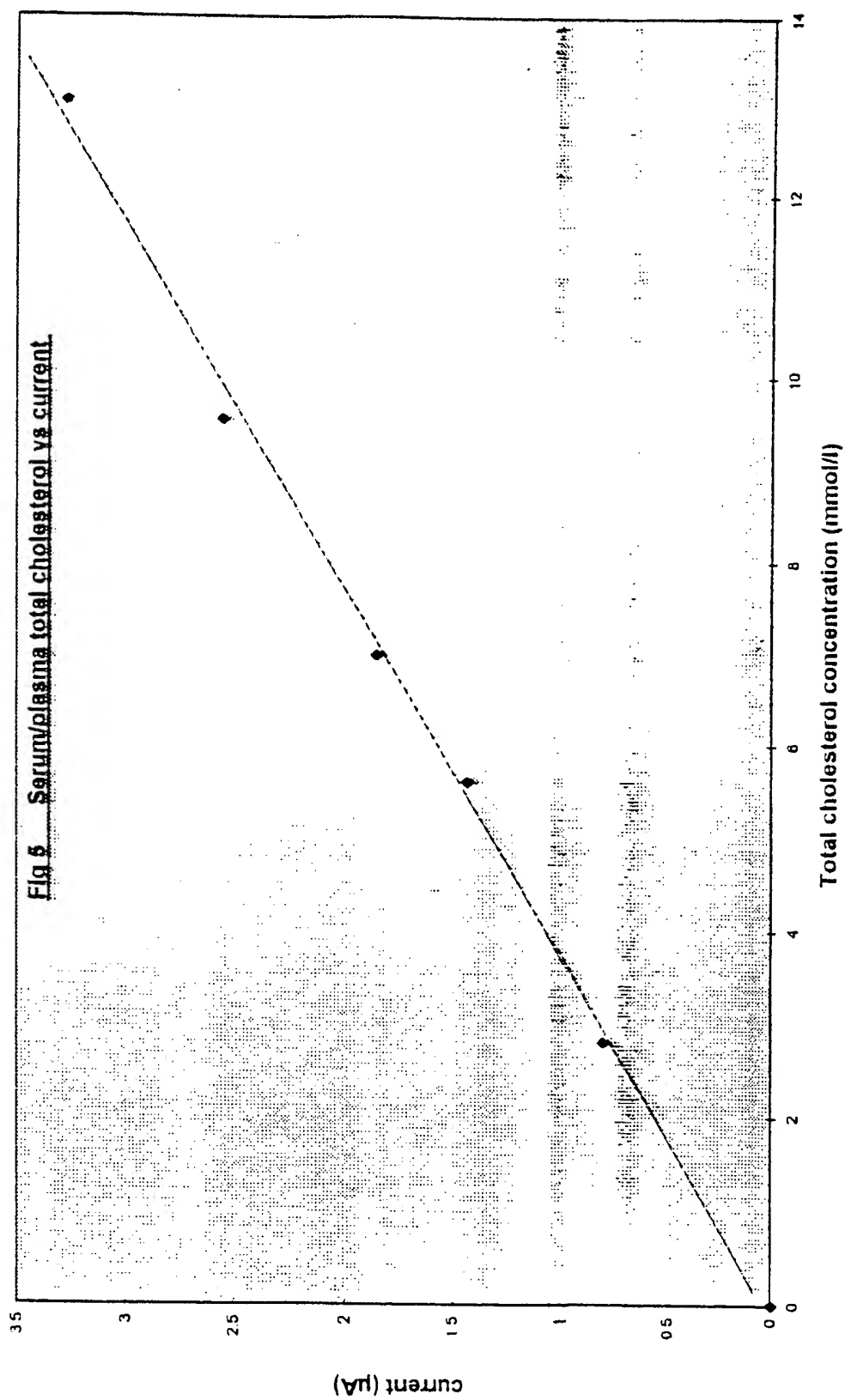


FIG. 4(d)

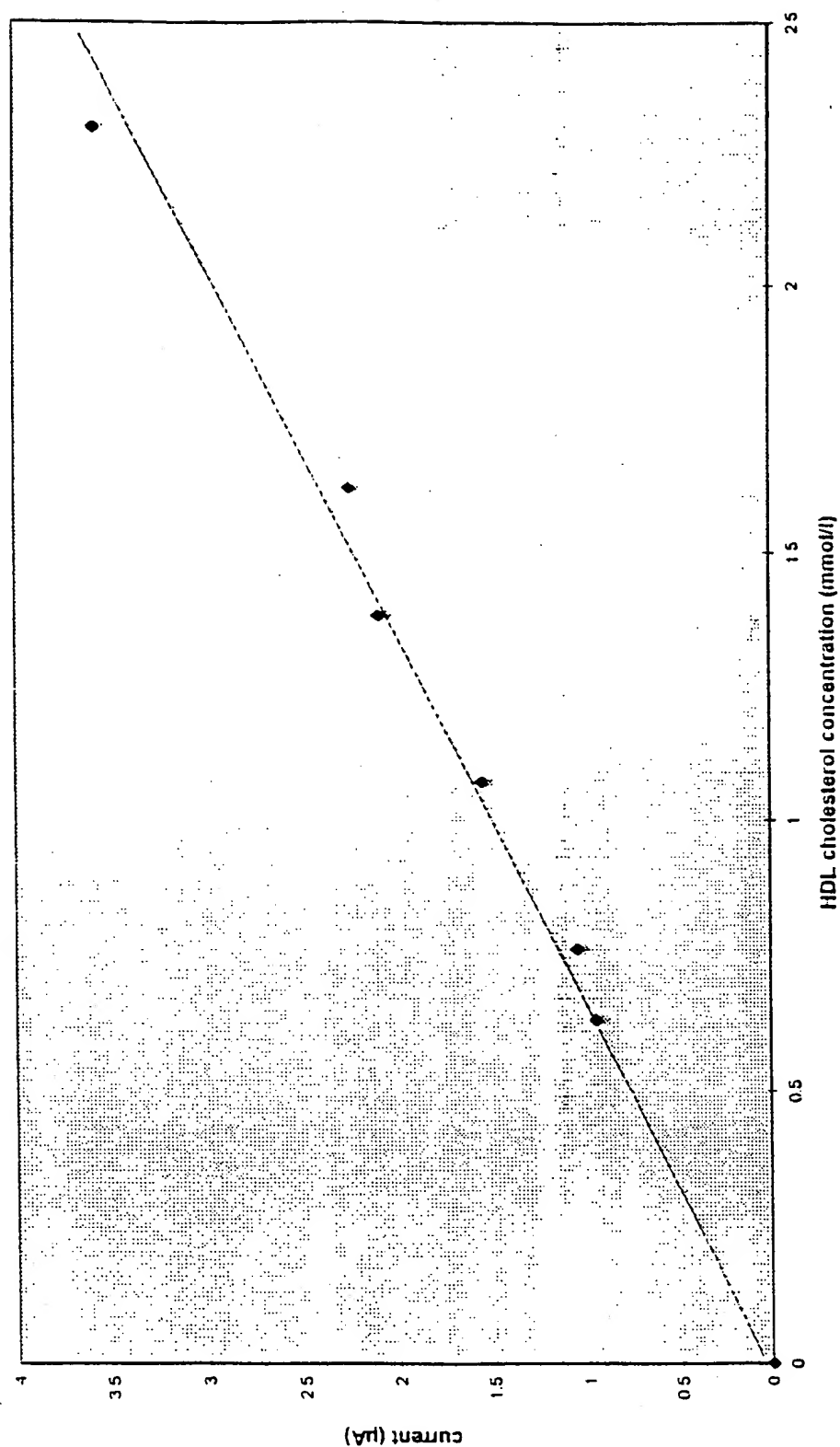


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Fig 6 Serum/plasma HDL cholesterol vs current



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/IE 98/00002

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC 6 G01N27/327 G01N33/543 C12Q1/00

According to International Patent Classification(IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 G01N C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category * | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|------------|---|-----------------------|
| A          | N. A. MORRIS ET AL: "An electrochemical capillary fill device for the analysis of glucose incorporating glucose oxidase and ruthenium (III) hexamine as mediator." ELECTROANALYSIS, vol. 4, no. 1, 1992, pages 1-9, XP002065769 see the whole document<br>--- | 1,17                  |
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Further documents are listed in the continuation of box C.



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Date of the actual completion of the international search

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## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages               | Relevant to claim No. |
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